Data Evaluation Report on the acute toxicity of "RIMON" 10EC (formulated product of Novaluron) on the Freshwater Alga *Selenastrum capricornutum*

PMRA Submission #:{.....} EPA MRID #: 45638411

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D285479
OECD Data Point	{.....}
EPA MRID	45638411
EPA Guideline	123-2

Test material: "RIMON" 10EC Purity: 9.1%

Common name: Not reported

Chemical name: IUPAC: 1,(3-chloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy)phenyl)-3-(2,6-difluorobenzoyl)urea (chemical name of active)
CAS name: Not reported
CAS No.: Not reported
Synonyms: formulation product of "RIMON"

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/1/03

QC Reviewer: Dana Worcester
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/1/03

Primary Reviewer: Bill Evans
{EPA/OECD/PMRA}

Date: {.....} 11/26/03

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 124002

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Jenkins, C.A. 1998. "RIMON" 10EC: Algal Growth Inhibition Assay. Unpublished study performed by Huntingdon Life Sciences Ltd, Suffolk, England. Laboratory Project Identification No. MAK/464/982147. Study submitted by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel. Sponsor Study No. R-9914. Experimental start date December 8, 1997 and experimental termination date December 12, 1997. The final report issued August 20, 1998.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of *Selenastrum capricornutum* were exposed to Novaluron ("RIMON" 10EC) under static conditions. The nominal concentrations were 15.5, 34.2, 75.1, 165, 364, and 800 mg/L. The mean measured concentrations were not detected (control), 10.2, 27.2, 66.2, 154, 332, and 765 mg/L. The cell density was reduced 6.5, 11, 84, 99, 99, and 99% in the 10.2, 27.2, 66.2, 154, 332, and 765 mg/L treatment groups, respectively. The growth rates were reduced 1, 2, 36, 85, 94, and 95% in the 10.2, 27.2, 66.2, 154, 332, and 765 mg/L treatment groups, respectively. The biomass (area under the growth curve) was reduced 13, 37, 88, 99, 100, and 100% in the 10.2, 27.2, 66.2, 154, 332, and 765 mg/L treatment groups, respectively. Biomass was the most sensitive endpoint; the EC_{50} was 32 mg/L. All endpoints were significantly reduced at the 66.2 mg/L treatment level. While the reduction in biomass (37%) was not detected to be statistically significant, it was considered to be biologically significant, so the NOEC for "RIMON" 10EC was determined to be 10.2 mg/L.

The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular Tier 2 plant study with *Selenastrum capricornutum* [§122-3]. However, since the required light intensity measurements of 4-5 Klux (±15%) were not used for the test, this study must be classified as Supplemental.

Results Synopsis

Test Organism: *Selenastrum capricornutum*
Test Type: Static

Cell Density:

NOEC: 27.2 mg/L	LOEC: 66.2 mg/L
EC_{50}: 39 mg/L	95% C.I.: 16-95 mg/L
Probit: 3.07 ± 1.36	

Growth rate:

NOEC: 27.2 mg/L	LOEC: 66.2 mg/L
EC_{50}: 74 mg/L	95% C.I.: 53-100 mg/L
Probit: 2.13 ± 0.259	

Area Under the Growth Curve (Biomass):

NOEC: 10.2 mg/L	LOEC: 66.2 mg/L
EC_{50}: 32 mg/L	95% C.I.: 17-59 mg/L
Probit: 3.30 ± 1.09	

Endpoint(s) Affected: Cell density, growth rate and biomass

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Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D285479
	OECD Data Point	{.....}
	EPA MRID	45638411
	EPA Guideline	123-2

Test material:	"RIMON" 10EC Purity: 9.1%
Common name:	Not reported
Chemical name:	IUPAC: 1,(3-chloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy)phenyl)-3-(2,6-difluorobenzoyl)urea (chemical name of active) CAS name: Not reported CAS No.: Not reported Synonyms: formulation product of "RIMON"

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan
Date: 4/1/03

QC Reviewer: Dana Worcester
Staff Scientist, Dynamac Corporation

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Primary Reviewer: Bill Evans
{EPA/OECD/PMRA}

Date: {.....}

Secondary Reviewer(s):{.....}
{EPA/OECD/PMRA}

Date: {.....}

Company Code	{.....}	[For PMRA]
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EPA PC Code	124002	

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OECD Procedure 201 (1984); EC Directive 92/69/EEC, Part C3 (1992); and U.S. EPA FIFRA Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget plants, §122-3h. The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The technical grade of the test chemical was not tested.
2. The light intensity was 6.420-6.5 Klux, EPA requires that the intensity be maintained at 4-5 Klux (±15%),
3. The length of the acclimation period and age of the inoculum were not specified.
4. The material of the test vessels was not reported.
5. The dilution water was dechlorinated. The pH of the dilution water was not reported.
6. The agitation rate of 150 rpm was greater than recommended (100 rpm).

These deviations did not affect the acceptability or the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material "RIMON" 10EC

Description: Clear liquid

Lot No./Batch No. : 960917

Purity: 9.1%

Stability of Compound
Under Test Conditions: The measured concentrations of Novaluron ("RIMON" 10EC) were 75-90% of nominal at hour 0 and 57-102% of nominal at hour 96. The 96 hour measured concentrations were 76-113% of 0 hour measured concentration (Table 1, p. 21).

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test material was stored at 4°C in the dark.

2. Test organism:
Name: *Selenastrum capricornutum*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S.

costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: CCAP number 278/4
Source: Originally from Culture Collection of Algae and Protozoa (CCAP), Freshwater Biological Association, Ferry House, Ambleside, Cumbria, UK. In-house laboratory cultures.
Age of inoculum: Several days old.
Method of cultivation: Sterile OECD medium (Appendix 2, p. 25)

B. STUDY DESIGN:

a) Range-finding Study: A range-finding study with Novaluron ("RIMON" 10EC) was conducted in order to estimate the nominal test concentrations for the definitive study. The range-finding test concentrations were 1, 10, 100, and 1000 mg/L. The results were not reported.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	Several days Sterile OECD medium (Appendix 2, p. 25); same as test. Not reported	The length of the acclimation period was not specified. *EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system static/static renewal: renewal rate for static renewal:	Static	
Incubation facility	Incubator	

Parameter	Details	Remarks *Criteria*
Duration of the test	96 hours	*EPA requires: 96 - 120 hours* *OECD: 72 hours*
Test vessel material: (glass/polystyrene) size: fill volume:	Sterilized conical flasks with non-absorbent cotton wool plugs 250 mL 50 mL	The material of the test vessels was not reported. *OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity **(for marine algae):**	Sterile OECD medium 6.6-8.2 7.4-7.9 $Na_2EDTA{\cdot}2H_2O$ (0.1 mg/L) $NaHCO_3$ (50.0 mg/L) N/A	*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP medium and no chelators.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	N/A	
Dilution water source: type: pH: salinity **(for marine algae):** water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Laboratory tap water Filtered, dechlorinated Not reported N/A Reverse osmosis Not reported Not reported Not reported Not reported Not reported	The dilution water was dechlorinated. The pH of the dilution water was not reported. *EPA pH: <u>Skeletonema costatum</u>= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*

Parameter	Details	Remarks *Criteria*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 150 rpm	The agitation rate of 150 rpm was greater than recommended (100 rpm). *EPA recommends agitation only for Selenastrum at 100 cycles per min and Skeletonema at ~60 cycles per min. Aeration is not recommended.*
Initial cells density	Approximately 10,000 cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Selenastrum capricornutum, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates control: solvent control: treated ones:	 6 N/A 3	One extra replicate in the control and treatment groups were used for environmental measurements and chemical analysis (not incubated). *EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test cultures should be included in the test.*

Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	control, 15.5, 34.2, 75.1, 165, 364, and 800 mg/L not detected (control), 10.2, 27.2, 66.2, 154, 332, and 765 mg/L	The mean measured concentration was determined from 0 and 96 hour mean recoveries (Table 1, p. 21). *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; 0 and 96 hours	
Test conditions temperature: photoperiod: light intensity and quality:	 22.6-24.4°C Continuous 6452 lux, cool-white fluorescent lighting	*EPA temperature: Skeletonema: 20°C, Others: 24-25°C; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical {if used) name: concentrations:	 Potassium dichromate Not reported	72 hour biomass EC_{50} was 0.52 mg/L (95% confidence limits 0.42 and 0.66 mg/L).
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell count, growth rate, and area under the growth curve	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Haemocytometer	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Every 24 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was exponential growth in the control	Yes, dilution water control group cell density at test termination was 156X greater than the dilution water control group cell density at test initiation.	*EPA requires control cell count at termination to be ≥2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

The cell density was 6.5, 11, 84, 99, 99, and 99% reduced in the 10.2, 27.2, 66.2, 154, 332, and 765 mg/L treatment groups, respectively. The growth rates were 1, 2, 36, 85, 94, and 95% reduced in the 10.2, 27.2, 66.2, 154, 332, and 765 mg/L treatment groups, respectively. The biomass (area under the growth curve) was 13, 37, 88, 99, 100, and 100% reduced in the 10.2, 27.2, 66.2, 154, 332, and 765 mg/L treatment groups, respectively. The percent reductions in growth rate and biomass were statistically significant in the 66.2, 154, 332, and 765 mg/L treatment groups.

Table 3: Effect of "RIMON" 10EC on freshwater alga (*Selenastrum capricornutum*)

Treatment mean measured and nominal concentrations [a] (mg/L)	Initial cell density (cells/mL)	Mean Cell density (cells/mL) at		
		24 hours	96 hours	
			cell count	% inhibition[b]
Dilution water control	~10,000	48,600	1,560,000	--
Solvent control	N/A	N/A	N/A	N/A
10.2 (15.5)	~10,000	48,400	1,460,000	6.5
27.2 (34.2)	~10,000	34,600	1,390,000	11
66.2 (75.1)	~10,000	22,500	254,000	84
154 (165)	~10,000	13,400	21,300	99
332 (364)	~10,000	9,170	13,800	99
765 (800)	~10,000	9,180	13,000	99
Reference chemical (if used)	N/A	N/A	N/A	N/A

[a] The nominal test concentrations are presented in parentheses.

[b] The % inhibition was reviewer-calculated from mean cell density data compared to the control.

Table 4: Effect of "RIMON" 10EC on the freshwater alga *Selenastrum capricornutum*

Mean Measured and Nominal Treatment Concentrations [a] (mg/L)	Initial cell density (cells/mL)	Mean Growth Rate per day ($\times 10^{-2}$)	% inhibition (Mean Growth Rate per day)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[b]
Dilution water control	~10,000	5.224	--	48,390,000	--
Solvent control	N/A	N/A	--	N/A	--
10.2 (15.5)	~10,000	5.154	1	41,910,000	13

Mean Measured and Nominal Treatment Concentrations [a] (mg/L)	Initial cell density (cells/mL)	Mean Growth Rate per day (x 10^{-2})	% inhibition (Mean Growth Rate per day)[b]	Mean Area Under Growth Curve	% inhibition (Mean Area Under Growth Curve)[b]
27.2 (34.2)	~10,000	5.116	2	30,590,000	37**
66.2 (75.1)	~10,000	3.355	36*	5,940,000	88*
154 (165)	~10,000	0.778	85*	487,000	99*
332 (364)	~10,000	0.330	94*	76,000	100*
765 (800)	~10,000	0.266	95*	56,900	100*
Reference chemical (if used)	Not reported	Not reported	Not reported	Not reported	Not reported

[a] The nominal test concentrations are presented in parentheses.
[b] The % inhibition is compared to the control.
*Statistically significant reduction compared to the control ($p<0.05$).
** Biologically significant reduction compared to the control.

Table 5: Statistical endpoint values.

Statistical Endpoint	Biomass	Growth rate	Cell density
NOEC or EC_{05} (mg/L)	10.2	27.2	Not reported
EC_{50} (mg/L)	33.5	87.2	Not reported
IC_{50} or EC_{50} (mg/L) (95% C.I.)	29.0-37.6	73.6-103	Not reported
other (IC_{25}/EC_{25})	Not reported	Not reported	Not reported
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

N/A = Not applicable.

B. REPORTED STATISTICS:

Statistical Method: The formulas used to calculate the area under the growth curve and growth rates are presented on pp. 13-14. The Dunnett's multicomparison test (multiple t-test) was used to compare the treatment group to the control and to determine the NOEC. The EC_{50} for growth rates and biomass were calculated by the Stephan computer program using percent inhibitions. The reported statistics were based on the measured test concentrations.

Cell Density:
NOEC: Not reported

EC_{50}: Not reported

Growth rate:
NOEC: 27.2 mg/L
EC_{50}: 87.2 mg/L 95% C.I.: 73.6-103 mg/L

Area Under the Growth Curve (Biomass):
NOEC: 10.2 mg/L
EC_{50}: 33.5 mg/L 95% C.I.: 29.0-37.6 mg/L

Endpoint(s) Affected: Growth rate and biomass

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Cell density, growth rate, and area under the growth curve (biomass) data were analyzed. The data for cell density and biomass were ln transformed to satisfy the assumptions of normality and homogeneity of variances; data for growth rate satisfied these assumptions without transformation. The NOEC and LOEC for all endpoints were determined using ANOVA, followed by William's test via TOXSTAT statistical software. The EC_{50} for all endpoints were determined using the probit method via Nuthatch statistical software.

Cell Density:
NOEC: 27.2 mg/L LOEC: 66.2 mg/L
EC_{50}: 39 mg/L 95% C.I.: 16-95 mg/L
Probit: 3.07 ± 1.36

Growth rate:
NOEC: 27.2 mg/L LOEC: 66.2 mg/L
EC_{50}: 74 mg/L 95% C.I.: 53-100 mg/L
Probit: 2.13 ± 0.259

Area Under the Growth Curve (Biomass):
NOEC: 10.2 mg/L LOEC: 66.2 mg/L
EC_{50}: 32 mg/L 95% C.I.: 17-59 mg/L
Probit: 3.30 ± 1.09

Endpoint(s) Affected: Cell density, growth rate and biomass

D. STUDY DEFICIENCIES:

The deviations did not affect the acceptability or validity of the study.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding the most sensitive endpoint, biomass, were identical to the study author's. The NOEC for this endpoint was determined to be 10.2 mg/L because, while reductions at the 27.2 mg/L treatment level were not detected to be statistically significant, they were considered to be biologically significant. Furthermore, the reviewer analyzed cell density data, while the study author did not. The reviewer's EC_{50} estimate for biomass was slightly lower

than that estimated by the study author because of the statistical methods used to determine this value. The reviewer's results are reported in the Executive Summary and Conclusions sections.

The 154, 332, and 765 mg/L test cultures were hazy emulsions on the day of preparation.

F. CONCLUSIONS: The study is scientifically sound and satisfies the guidelines for an aquatic nonvascular Tier 2 plant study with *Selenastrum capricornutum* [§122-3]. However, since the required light intensity measurements of 4-5 Klux (±15%) were not used for the test, this study must be classified as Supplemental. Biomass was the most sensitive endpoint; the EC_{50} was 32 mg/L. All endpoints were significantly reduced at the 66.2 mg/L treatment level. While the reduction in biomass (37%) was not detected to be statistically significant, it was considered to be biologically significant, so the NOEC for "RIMON" 10EC was determined to be 10.2 mg/L.

Cell Density:
NOEC: 27.2 mg/L LOEC: 66.2 mg/L
EC_{50}: 39 mg/L 95% C.I.: 16-95 mg/L
Probit: 3.07 ± 1.36

Growth rate:
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Probit: 3.30 ± 1.09

Endpoint(s) Affected: Cell density, growth rate and biomass

III. REFERENCES:

US EPA Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 122 Tier 1 of Non-target area testing, 122-30 (e) *Selenastrum capricornutum*: growth conditions, October 1982.

OECD Guidelines for Testing of Chemicals, Procedure 201. "Alga, Growth Inhibition Test", adopted 7 June, 1984.

Official Journal of the European Communities. L383A. Part C: Methods for Determination of Ecotoxicity: C.3. Algal Inhibition Test. Vol 35, 29 December 1992. ISSN 0378-6978.

Stephan, C.E. (1977). Methods for Calculating an LC_{50}. Aquatic Toxicology and Hazard Evaluation. ASTM STP 634.

Stephan, C.E. (1982) A computer program for calculating an LC_{50}. US Environmental Protection Agency.

Dunnett, C.W. (1955) A multiple comparison procedure for comparing several treatments with a control. Journal of American Statistical Association, 50, 1096-1121.

Dunnett, C.W. (1964) New tables for multiple comparisons with a control. Biometrics, 20, 482-491.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

cell density
File: 8411ce Transform: NATURAL LOG(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	106.210	17.702	310.561
Within (Error)	17	0.967	0.057	
Total	23	107.177		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

cell density
File: 8411ce Transform: NATURAL LOG(Y)

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	5.015	156.217		
2	10.2	4.948	145.667	0.397	
3	27.2	4.912	139.333	0.612	
4	66.2	3.221	25.400	10.629	*
5	154	0.747	2.127	25.282	*
6	332	0.317	1.377	27.829	*
7	765	0.255	1.297	28.193	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

cell density
File: 8411ce Transform: NATURAL LOG(Y)

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	10.2	3	54.423	34.8	10.550
3	27.2	3	54.423	34.8	16.883
4	66.2	3	54.423	34.8	130.817
5	154	3	54.423	34.8	154.090
6	332	3	54.423	34.8	154.840
7	765	3	54.423	34.8	154.920

cell density
File: 8411ce Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	156.217	5.015	5.015
2	10.2	3	145.667	4.948	4.948
3	27.2	3	139.333	4.912	4.912
4	66.2	3	25.400	3.221	3.221
5	154	3	2.127	0.747	0.747
6	332	3	1.377	0.317	0.317
7	765	3	1.297	0.255	0.255

cell density
File: 8411ce Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	5.015				
10.2	4.948	0.397		1.74	k= 1, v=17
27.2	**4.912**	**0.613**		**1.82**	**k= 2, v=17**
66.2	3.221	10.638	*	1.85	k= 3, v=17
154	0.747	25.302	*	1.87	k= 4, v=17
332	0.317	27.851	*	1.87	k= 5, v=17
765	0.255	28.215	*	1.88	k= 6, v=17

s = 0.239
Note: df used for table values are approximate when v > 20.

growth rate
File: 8411gr Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	115.246	19.208	309.806
Within (Error)	17	1.049	0.062	
Total	23	116.296		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

growth rate
File: 8411gr Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	5.224	5.224		
2	10.2	5.154	5.154	0.396	

3	27.2	5.116	5.116	0.612	
4	66.2	3.355	3.355	10.615	*
5	154	0.778	0.778	25.252	*
6	332	0.330	0.330	27.796	*
7	765	0.266	0.266	28.158	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

growth rate
File: 8411gr Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	10.2	3	0.467	8.9	0.070
3	27.2	3	0.467	8.9	0.108
4	66.2	3	0.467	8.9	1.869
5	154	3	0.467	8.9	4.446
6	332	3	0.467	8.9	4.894
7	765	3	0.467	8.9	4.958

growth rate
File: 8411gr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	6	5.224	5.224	5.224
2	10.2	3	5.154	5.154	5.154
3	27.2	3	5.116	5.116	5.116
4	66.2	3	3.355	3.355	3.355
5	154	3	0.778	0.778	0.778
6	332	3	0.330	0.330	0.330
7	765	3	0.266	0.266	0.266

growth rate
File: 8411gr Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	5.224				
10.2	5.154	0.397		1.74	k= 1, v=17
27.2	**5.116**	**0.613**		**1.82**	**k= 2, v=17**
66.2	3.355	10.639	*	1.85	k= 3, v=17
154	0.778	25.309	*	1.87	k= 4, v=17
332	0.330	27.859	*	1.87	k= 5, v=17
765	0.266	28.221	*	1.88	k= 6, v=17

s = 0.248
Note: df used for table values are approximate when v > 20.

biomass
File: 8411b Transform: NATURAL LOG(Y)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	186.855	31.143	164.778
Within (Error)	17	3.220	0.189	
Total	23	190.076		

Critical F value = 2.70 (0.05,6,17)
Since F > Critical F REJECT Ho:All groups equal

biomass
File: 8411b Transform: NATURAL LOG(Y)

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	8.425	4839.167		
2	10.2	8.310	4190.667	0.372	
3	27.2	8.017	3059.333	1.327	
4	66.2	6.375	594.000	6.670	*
5	154	3.844	48.733	14.901	*
6	332	2.014	7.600	20.856	*
7	765	1.461	5.687	22.653	*

Bonferroni T table value = 2.65 (1 Tailed Value, P=0.05, df=17,6)

biomass
File: 8411b Transform: NATURAL LOG(Y)

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	6			
2	10.2	3	2543.235	52.6	648.500
3	27.2	3	2543.235	52.6	1779.833
4	66.2	3	2543.235	52.6	4245.167
5	154	3	2543.235	52.6	4790.433
6	332	3	2543.235	52.6	4831.567
7	765	3	2543.235	52.6	4833.480

biomass
File: 8411b Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN

1	control	6	4839.167	8.425	8.425
2	10.2	3	4190.667	8.310	8.310
3	27.2	3	3059.333	8.017	8.017
4	66.2	3	594.000	6.375	6.375
5	154	3	48.733	3.844	3.844
6	332	3	7.600	2.014	2.014
7	765	3	5.687	1.461	1.461

biomass
File: 8411b Transform: NATURAL LOG(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	8.425				
10.2	8.310	0.372		1.74	k= 1, v=17
27.2	**8.017**	**1.325**		**1.82**	**k= 2, v=17**
66.2	6.375	6.662	*	1.85	k= 3, v=17
154	3.844	14.884	*	1.87	k= 4, v=17
332	2.014	20.832	*	1.87	k= 5, v=17
765	1.461	22.628	*	1.88	k= 6, v=17

s = 0.435
Note: df used for table values are approximate when v > 20.